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Impact of nest sanitation behavior on hosts' egg rejection: an empirical study and meta-analyses

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Abstract

Egg rejection in birds is a specific adaptation toward avian brood parasitism, whereas nest sanitation is a general behavior for cleaning the nest and avoiding predation. However, both behaviors refer to the action of ejecting objects out of the nest, and nest sanitation has been proposed as a pre-adaptation for egg rejection. Here, we tested the eliciting effect of nest sanitation on egg rejection in the red-whiskered bulbul *Pycnonotus jocosus*, a potential host species that are sympatric with parasitic cuckoos. We conducted meta-analyses of previous studies on both nest sanitation and egg rejection, in order to evaluate the consistency of our conclusions. Our results showed that nest sanitation did not elicit egg rejection in *P. jocosus*. The conclusions concerning such an eliciting effect from previous studies were mixed, whereas the methodologies were inconsistent, making the studies unsuitable for comparisons. However, the ejection frequency of nest sanitation was consistently higher than the frequency of egg rejection across different host species or populations. These results suggest that nest sanitation, which is an ancient behavior, is more fundamental than egg rejection, but the effect of the former on the latter is complex and needs further study. Standardized methodologies and the integration of behavior, physiology, and modeling may provide better opportunities to explore the relationship between nest sanitation and egg rejection.

Key words: avian brood parasitism, egg discrimination, nest cleaning, pre-adaptation, *Pycnonotus jocosus*.

Avian brood parasitism (ABP) is an important topic in the fields of biological evolution and animal cognition (Soler 2017). According to the behavior of the parasites, brood parasitism has been divided into 2 branches, interspecific (including facultative and obligate brood parasitism) and conspecific (Lanyon 1992; Lyon and Eadie 2008; Shizuka and Lyon 2010; Feeney et al. 2014; Mann 2017). To date, the related reports of obligate ABP mainly include 5 taxa, covering 107 species of birds (Lowther 2020). The co-evolutionary relationship between hosts and parasites is considered an ideal model for exploring avian cognition and learning (Rothstein 1990) because natural selection favors the hosts with the ability to evolve capacity for recognizing parasites (i.e., parasite adults, eggs, or nestlings) as anti-parasitic adaptations (Davies 2000; Soler 2017). Egg recognition is one of the most effective and important defenses that allow hosts to visit their nests a sufficient number of times for discrimination to occur; however, egg recognition does not always occur through the action of rejection (Lyon 2003; Antonov et al. 2008; Feeney et al. 2014; Ruiz-Raya et al. 2015). The egg rejection behavior of hosts is a flexible phenotypic character with the ability to vary across populations of a single species, or within different stages of reproduction in a single population (Moskát 2005; Yang et al. 2015a, 2020a). The potential effects of phenotypic plasticity are not only manifest in both spatial and geographic variations, but also in breeding seasonal changes and the genetic component of egg recognition of hosts (reviewed in Ruiz-Raya and Soler 2017). Because of the unusually strong eggshells of parasite eggs (Soler et al. 2019), host egg rejection can be achieved by different means, such as ejecting, puncturing, grasping, burying, and nest desertion, depending on the bill size of the hosts (Bártol et al. 2003; Antonov et al. 2008). Fundamentally, many factors influence egg rejection performance in brood parasite hosts.

Other than egg rejection, recognition related to nest content also includes egg retrieval and nest sanitation (Guigueno and Sealy 2012; Yang et al. 2015a, 2015b, 2019a; Lyon and Shizuka 2017). Nest sanitation refers to the removal of a variety of objects from nests such as dropping vegetation, egg shells, fecal sacs, invertebrate parasites, occasionally dead nestlings, and unhatched eggs by parents; therefore, its behavioral pattern is similar to that of egg ejection (reviewed in Guigueno and Sealy 2012; Luro and Hauber 2017). However, nest sanitation is more common and lasts throughout the entire breeding period, and its goal is to create a clean and tidy environment for offspring and to avoid attracting predators. As a result, Rothstein (1975) proposed the hypothesis that nest sanitation is a pre-adaption for egg rejection. Then, based on this hypothesis, a hierarchical concept in which hosts reject non-egg-shaped objects as a general cleaning mechanism and discriminate egg-shaped objects as an adaptation was proposed (Moskát et al. 2003). This was supported by some studies (Guigueno and Sealy 2012; Yang et al. 2015a), and further research found that sanitation behavior could promote the occurrence of egg rejection in hosts (hereinafter rejection promoting hypothesis) (Yang et al. 2015b; Feng et al. 2019). Alternatively, the rejection rate of egg-shaped objects increased with that of nest sanitation (non-egg-shaped objects) in hirundines among different populations, which indicated a reduction in nest parasitism costs, suggesting that egg recognition ability might evolve from the recognition of non-egg-shaped objects (Yang et al. 2015b). However, some studies found no support for this promoting phenomenon (Peer and Sealy 2004; Luro and Hauber 2017; Su et al. 2018). Contrarily, the rejection of egg-shaped objects was highly repeatable within individuals yet variable between them. The rejection was neither influenced by non-egg-shaped objects nor related to nest sanitation in *Turdus migratorius*, and no significant correlation was found between nest sanitation and egg-shaped object ejection rates after comprehensive analysis of 8 host species from published data (Luro and Hauber 2017). Therefore, the nest sanitation hypothesis in host species posited a mixed conclusion that needs clarification with more research. Additionally, egg burying has been suggested as a behavior for cleaning nests rather than a strictly anti-parasitism strategy (Guigueno and Sealy 2009). Burying rejected eggs during nesting is likely to be a continuation of the nesting process rather than a true rejection of parasite eggs (Reel and Underwood 2019). Therefore, sanitation might be an exaptation for anti-parasite defense (Guigueno and Sealy 2012). Briefly, egg ejection and nest sanitation may be similar in action but different in cognition. This idea is still controversial and needs further study and discussion (Peer 2017).

In this study, we tested the egg promotion hypothesis in red-whiskered bulbuls *Pycnonotus jocosus*, a species reported as a host of the banded bay cuckoo *Cacomantis sonneratii* (Lowther 2020), by investigating the impact of nest sanitation on both the frequency and latency of egg rejection. We inserted either one single model egg or a model egg along with a peanut shell into the host nests; the former was aimed at testing the egg rejection of the hosts, whereas the later was intended to investigate the impact of nest sanitation on egg rejection. If nest sanitation can elicit egg rejection, it is expected that *P. jocosus* would reject parasite eggs more frequently when they are along with the peanut shell. We also investigated for the first time the latency to rejection by hosts, which had not been tested for this hypothesis before, and we predicted that *P. Jocosus* would reject parasite eggs more rapidly when they are along with the peanut shell. Moreover, considering the mixed conclusions from previous studies, we also performed meta-analyses of the results of this study and previous studies, in order to explore a more robust conclusion.

Materials and Methods

Study area and species

This study was performed during the breeding season (April to August in 2019–2020) in a village named Nonggang, Guangxi Province, southwestern China (23°39′N, 107°04′E; Bi et al. 2020). *Pycnonotus jocosus* is a local resident species sympatric with several cuckoo species (Yang et al. 2012); the clutch size, mean weight, length, and width of the *P. jocosus* were 2.82 ± 0.51, 2.63 ± 0.30 g, 21.15 ± 1.35 mm, and 15.62 ± 0.69 mm, respectively ($n = 105$); and the hatching period is 10–12 days. However, no cuckoo parasitism has been found in this host population.

Experimental procedures for egg rejection and nest sanitation

Natural *P. jocosus* nests were found by searching the breeding habitat. The nests were randomly divided into 3 groups: (1) a single model egg group, in which one single blue model egg (ca. 21.1×15.6 mm) of polymer clay was inserted into host nests; (2) a combined group, in which one blue model egg and one half of a peanut shell were inserted into host nests; and (3) a control group, in which the manipulation procedure was the same, but no object was inserted into the nests. Manipulation of host nests was conducted after clutch completion during early incubation, and then observed nests were checked every 24 h after manipulation until the sixth day to confirm the hosts' responses and the latency of their reaction. Inserted objects were accepted by hosts when they were kept warm together with host eggs, or rejected by hosts when they were pecked (investigated by pecking marks on model eggs), ejected, buried, or deserted. To minimize the disturbance during checking, a nonintrusive thermal imaging camera (FLIR ONE PRO, FLIR Systems Inc., Sweden) was used to examine the incubation status.

Statistical analyses of experimental data

Generalized linear mixed models (GLMMs) with binomial responses (acceptance or rejection) were used to analyze the host responses to experimental manipulation. Treatment (single model egg or combined group) was considered as a fixed effect, whereas nest identity was entered as a random effect. Furthermore, the effects of the clutch size and egg-laying date were also tested in the GLMMs. The Cox regression was used to compare the latency to rejection, and chi-square tests were used to compare the rejection rates between model eggs and peanut shells within the combined group. In the Cox regression model, survival time (6 days with an interval every 24 h) of foreign objects (model egg/peanut shell) was the dependent variable with acceptance or rejection as status (an event was defined as an occurrence when rejection occurred), and treatments were included as the categorical covariate. Cox regression assumes that all survival functions have the same shape (Stevens et al. 2008), and so checking the foreign objects at regular and constrained time intervals (i.e., every 24 h) ensures that this assumption is valid in this form of experiment. Statistical analyses were performed using SPSS version 25.0 for Windows (IBM Inc., Armonk, NY). All tests were 2-tailed, and the significance level P-value was set to 0.05.

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Review and meta-analyses of previous studies

First, through the advanced retrieval mode of the Web of Science, the target research articles were filtered out according to the keywords "nest sanitation," "nest cleaning," "egg rejection," "egg recognition," "egg ejection," and "egg discrimination," combined with "AND" or "OR" and other command characters. Second, the studies that covered both egg rejection and nest sanitation were selected to the extract raw data of the experiments. For the 26 related papers extracted from the literature, only 10 (including this study) matched our research theme (i.e., both the nest sanitation and egg rejection behaviors of the hosts were tested), whereas others were filtered out. Combined with this study, raw data that covered 10 genera and 11 species of birds were extracted, and each research article was re-organized according to the tests for egg-shaped and non-egg-shaped objects. The research methods of the 2 articles among these studies were highly consistent with this study, based on the use of half peanut shells added as the non-egg-shaped objects in the experimental group (Yang et al. 2015b; Su et al. 2018). Data included the host species and its Latin name, the research year, the study site, the effective sample size, and the total sample sizes of the rejection and nest sanitation. The meta-analysis of previous studies for the effect of nest sanitation on egg rejection was performed using the *metabin* function in the *Meta* package of R (version 4.13-0). *Metabin* is a calculation of fixed and random effects for meta-analyses with binary outcome data. After that, a deviation analysis was carried out to test for heterogeneity using the *metabias* function. After collection and proofreading, the calculated original data in the similarity study were reclassified as egg-shaped objects (model eggs used to test egg rejection) and non-egg-shaped objects (objects used to test nest sanitation).

Ethical Note

The experiments reported here comply with the current laws of China. Experimental procedures were in agreement with the Animal Research Ethics Committee of Hainan Provincial Education Centre for Ecology and Environment, Hainan Normal University (permit no. HNECEE-2012-002).

Results

Fifty-five nests received treatment of either a single model egg ($n = 25$) or a model egg with a peanut shell ($n = 30$). *Pycnonotus jocosus* rejected the egg by ejection. We checked the surface integrity of model eggs that were not removed from nests by hosts and did not find any pecking marks in model eggs during the experiment. After excluding 7 nests that were predated during the experiment, the rejection rates of the model eggs in the single model egg group and combined group were 40.9% ($n = 22$) and 23.1% ($n = 26$), respectively. No rejection (ejection, desertion, or burial) was detected in the control group ($n = 25$). In the combined group, 100% of peanut shells were rejected, which was significantly higher than the 23.1% of the model eggs ($\chi^2 = 32.5$, $df = 1$, $P < 0.001$, chi-square test). GLMMs showed that the rejection rates of the model eggs did not differ between the treatment groups ($F_{1,44} = 0.725$, $P = 0.399$; Table 1). Neither the clutch size nor the egg laying date predicts the egg rejection rates (Table 1).

The rejection latency of the peanut shells in the combined group was significantly shorter than that of the model eggs in the combined group (Wald = 5.312, $df = 1$, $P = 0.021$, Cox regression; Figure 1) or in the single model egg group (Wald = 9.454, $df = 1$, $P = 0.002$,

Table 1. The results of the GLMM analysis for egg discrimination in *P. jocosus*

Effects	F	df1	df2	P-value
Treatment[a]	0.725	1	44	0.399
Clutch size	< 0.001	1	44	0.997
Egg laying date	1.716	1	44	0.197

[a] Treatment refers to the single model egg group or the combined group with a model egg and a peanut shell. Nest identity was included as a random effect.

Cox regression). However, the rejection latency of the model eggs in the single model group did not differ from that of the combined group (Wald = 0.668, $df = 1$, $P = 0.414$, Cox regression).

Nest sanitation behavior of non-egg-shaped objects (half of a peanut shell) may not increase the rate of egg rejection to some extent, and this was not statistically significant according to the results of the risk ratio (RR) from meta-analysis (random effects model: Z = 0.39; $P = 0.6992$; see Table 2 for details). There was no significant difference in the test for overall effect among these 3 studies ($Q_t = 5.45$, $df = 2$, $P = 0.0646$, Mantel–Haenszel method). In addition, a linear regression test of funnel plot asymmetry indicated that there was no heterogeneity among these 3 articles ($t = 0.37757$, $df = 1$, $P = 0.7702$, $K = 3$; Figure 3).

Further analyses including the 10 research studies listed above showed that the rejection frequencies of the egg-shaped and non-egg-shaped objects were significantly different (random effects model: Z = 5.08; $P < 0.0001$, see Table 3 for details). There was a significant difference in the test for overall effect among these studies ($Q_t = 103.78$, $df = 14$, $P < 0.0001$, Mantel–Haenszel method). The linear regression test of funnel plot asymmetry was carried out using the *metabias* function, indicating that there was some kind of a bias among these studies ($t = 2.1664$, $df = 13$, $P = 0.04945$, $K = 15$; Figure 4). In the parallel comparison between egg rejection and nest sanitation, 73.3% (11/15) of hosts that rejected the egg-shaped objects fell into the probability range of $[0 \leq P \leq 0.5]$ (Figure 2), whereas for the rejection rates of non-egg-shaped objects, 86.7% (13/15) of the hosts were in the probability range of $[0.5 < P \leq 1.0]$. Red-rumped swallows *Cecropis daurica* in northeast China and yellow warblers *Dendroica petechia* in Manitoba, Canada, fell <0.5 in range (Figure 2).

Discussion

Nest sanitation has been claimed to be an influencing factor that elicits egg rejection in hosts (Yang et al. 2015a), but the conclusions have been mixed (e.g., Guigueno and Sealy 2012; Yang et al. 2015b; Feng et al. 2019; but see Peer 2017; Luro and Hauber 2017; Su et al. 2018). However, methodologies vary among studies, which may restrict the feasibility for comparisons. For example, combining with those studies that were filtered out from meta-analyses but were related with this topic, inconsistencies were found in the timing of testing, egg types (real or model eggs; Yang et al. 2015a; Peer 2017; Yi et al. 2020), mimetic degree (Underwood and Sealy 2006; Zölei et al. 2012; Yang et al. 2019b), egg size and materials (Guigueno and Sealy 2009; Roncalli et al. 2016), egg colors (Feng et al. 2019), egg spots or patterns (Wang et al. 2015), or 3D or flat objects (Poláček et al. 2013). Furthermore, the shapes and materials used in detecting nest sanitation varied as well. The shapes of objects used in the experiments included stems, discs, coins, sticks (Moskát et al.
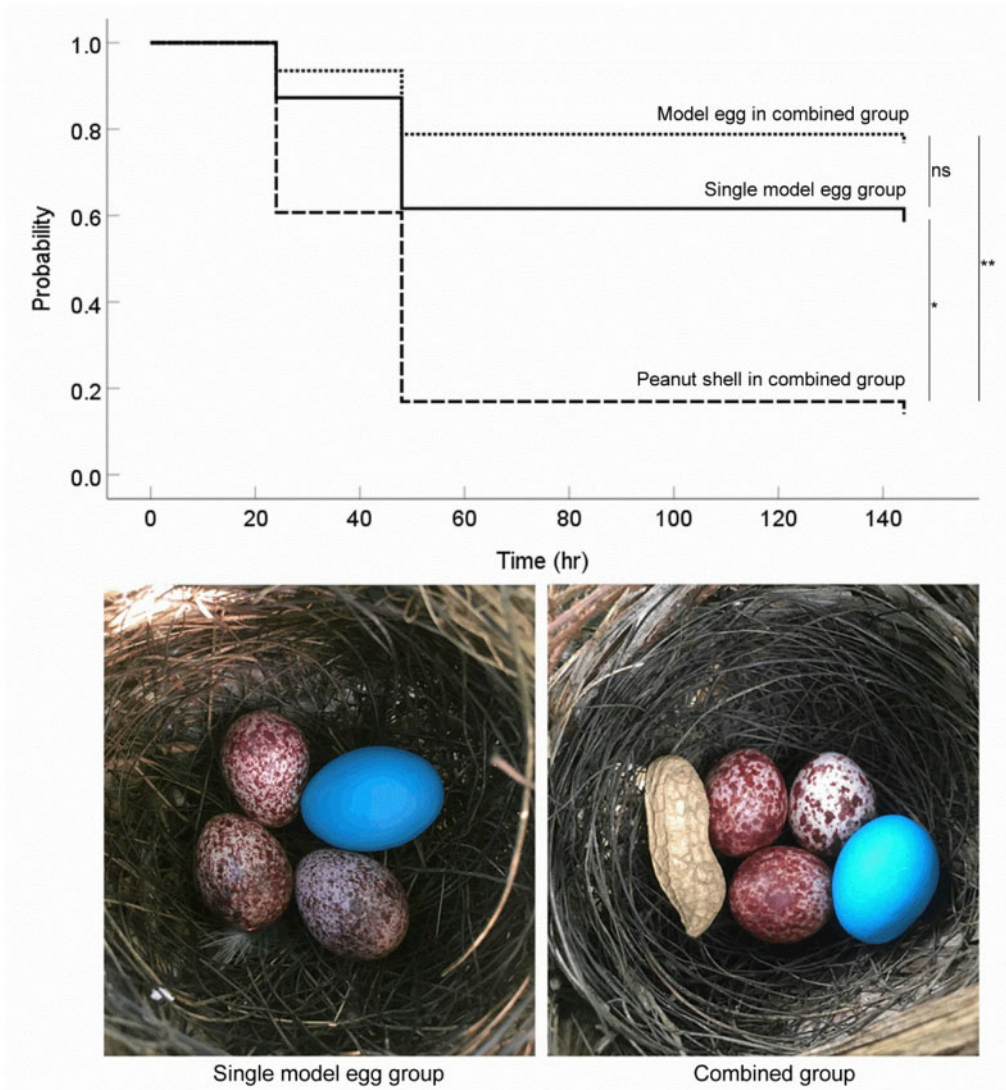
Figure 1. Comparison of latency to rejection by Cox regression between the treatment of a single model egg ($n = 25$) and a model egg with a peanut shell ($n = 30$) in *P. jocosus*.

Table 2. Meta-analysis of the effect of nest sanitation on egg rejection with consistent methods

Species	Model egg and peanut shell		Model egg		RR	95% CI	Weight (fixed) (%)	Weight (random)
	Effect size	Total size	Effect size	Total size				
Barn Swallow (northeast China)[a]	14	25	10	37	2.07	1.10–3.90	27.2	34.4
Brown-breasted Bulbul (southwest China)[b]	10	19	14	26	0.98	0.56–1.70	39.9	37.4
Red-whiskered Bulbul (southwest China)[c]	7	26	9	22	0.66	0.29–1.48	32.9	26.2
Fixed effect model ($z = 0.84$, $P = 0.3988$)	–	70	–	85	1.17	0.81–1.68	100.0	–
Random effect model ($z = 0.39$, $P = 0.6992$)	–	–	–	–	1.13	0.60–2.12	–	100.0
Heterogeneity: $I^2 = 63\%$, $\tau^2 = 0.1951$, $P = 0.06$	–	–	–	–	–	–	–	–

Note: [a]Yang et al., 2015a; [b]Su et al., 2018; [c]This study.

2003; Yang et al. 2015a; Huo et al. 2018), pom-poms, oblongs, stars, dumbbells (Ortega and Cruz 1988; Guigueno and Sealy 2009), peanut shells (Yang et al. 2015b; Su et al. 2018), natural or artificial debris (Peer 2017), and fir leaves (Luro and Hauber 2017). Therefore, a comprehensive analysis of these results may be limited.

According to the results of our empirical study, the rejection rate of model eggs in the combined group did not differ from that in the single model egg group, indicating that nest sanitation did not influence the egg rejection rate in *P. jocosus*. Meta-analyses revealed that the results from these 3 related studies supported this conclusion
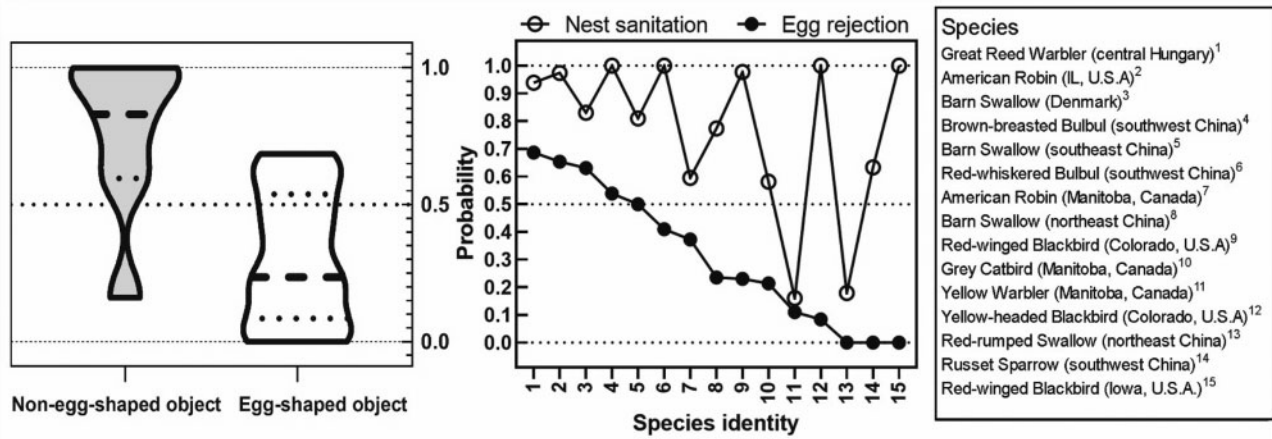
Figure 2. Probability and the distribution pattern of nest sanitation and egg rejection in hosts.

Table 3. Meta-analysis of related studies that tested the rejection of egg-shaped and non-egg-shaped objects in hosts

Species	Non-egg-shaped object		Egg-shaped object		RR	95% CI	Weight (fixed) (%)	Weight (random) (%)
	Effect size	Total size	Effect size	Total size				
Great Reed Warbler (central Hungary)[a]	90	96	35	51	1.37	1.13–1.66	20.0	9.5
American Robin (IL, USA)[b]	75	77	49	75	1.49	1.26–1.77	21.7	9.6
Barn Swallow (Denmark)[c]	39	47	17	27	1.32	0.96–1.81	9.5	8.9
Brown-breasted Bulbul (southwest China)[d]	19	19	14	26	1.83	1.29–2.58	5.4	8.8
Barn Swallow (southeast China)[e]	38	47	9	18	1.62	1.00–2.62	5.7	7.9
Red-whiskered Bulbul (southwest China)[f]	25	25	9	22	2.37	1.46–3.84	4.4	7.9
American Robin (Manitoba, Canada)[g]	25	42	16	43	1.60	1.01–2.54	6.9	8.1
Barn Swallow (northeast China)[h]	34	44	4	17	3.28	1.37–7.85	2.5	5.4
Red-winged Blackbird (Colorado, USA)[i]	43	44	41	178	4.24	3.23–5.57	7.1	9.2
Grey Catbird (Manitoba, Canada)[j]	25	43	9	42	2.71	1.44– 5.11	4.0	6.9
Yellow Warbler (Manitoba, Canada)[k]	46	287	22	200	1.46	0.91–2.34	11.4	8.0
Yellow-headed Blackbird (Colorado, USA)[l]	6	6	7	83	11.13	5.62–22.04	0.5	6.6
Red-rumped Swallow (northeast China)[m]	5	28	0	14	5.60	0.33– 94.38	0.3	1.0
Russet Sparrow (southwest China)[n]	19	30	0	21	27.49	1.75–431.05	0.3	1.1
Red-winged Blackbird (Iowa, USA)[o]	33	33	0	20	41.00	2.65–633.57	0.3	1.1
Fixed effect model ($z = 12.88$, $P < 0.0001$)		868	—	837	2.04	1.83– 2.27	100.0	—
Random effect model ($z = 5.08$, $P < 0.0001$)	—	—	—	2.31	1.71–3.13	—	100.00
Heterogeneity: $I^2 = 87\%$, $\tau^2 = 0.2397$, $P < 0.01$								

Note: (Moskát et al. 2003)[a]; (Luro and Hauber 2017)[b]; (Yang et al. 2015b)[c,e,h,m]; (Su et al. 2018)[d]; This study[f]; (Underwood and Sealy 2006)[g,j]; (Ortega and Cruz 1988)[i,l]; (Guigueno and Sealy 2009)[k]; (Huo et al. 2018)[n]; (Peer 2017)[o].

(see Table 2). However, our study surveyed the latency to rejection between nest sanitation and egg rejection, which, to our knowledge, had not been investigated before. The results showed that the latency to rejection for peanut shells was significantly shorter than that for model eggs, either in the single model egg group or in the combined group (Figure 1). This implied that *P. jocosus* made a quicker decision to eject a foreign non-egg-shaped object than to eject a foreign egg. One possible explanation is that the recognition of non-egg-shaped objects was easier than that of egg-shaped objects, and non-egg-shaped objects were easier for a grasp during rejection than were egg-shaped objects. Alternatively, egg rejection

involves a recognition process, whereas nest sanitation may be more instinctive, as in a fixed action pattern. All this implied that nest sanitation may be more fundamental in evolution and may act as a pre-adaptation for egg rejection behavior.

In contrast to the mixed conclusions for a promotion effect of nest sanitation on egg rejection, general analyses indicated that the hosts' rejection rates of non-egg-shaped objects were consistently higher than those of egg-shaped objects (Figure 2). Such a tendency existed both between different hosts species and between different populations within the same host species (Underwood and Sealy 2006; Yang et al. 2015a; Luro and Hauber 2017). These results

indicated that nest sanitation (i.e., ejecting non-egg-shaped objects) may be a more general, ancient behavior than egg rejection in evolution. However, publication bias was also found in these studies, probably due to the inconsistency of methodologies. For instance, studies were carried out in the pre-egg-laying stage (Bártol et al. 2003; Hoover 2003), the pre-incubation stage or after clutch completion (Huo et al. 2018; Yang et al. 2019a), the pre-incubation or incubation stage (Guigueno and Sealy 2009), the egg laying or incubation stage (Luro and Hauber 2017; Peer 2017), or in other types of mixed stages (Moskát et al. 2003; Moskát and Hauber 2007; Wang et al. 2015). Such differences might influence the results of hosts' recognition because the costs and benefits of nest sanitation and egg rejection could change with the changing stages of breeding. For example, nest sanitation might be more beneficial after clutch completion because foreign objects that fell into the nest cups would have a direct negative impact on egg incubation. Moreover, the co-evolution of hosts and parasites is not only affected by region, but also by time and the phylogeny of the species (Kruger 2007; Abolins-Abols and Hauber 2020). Furthermore, non-egg-shaped objects used to test nest sanitation also varied considerably from 1
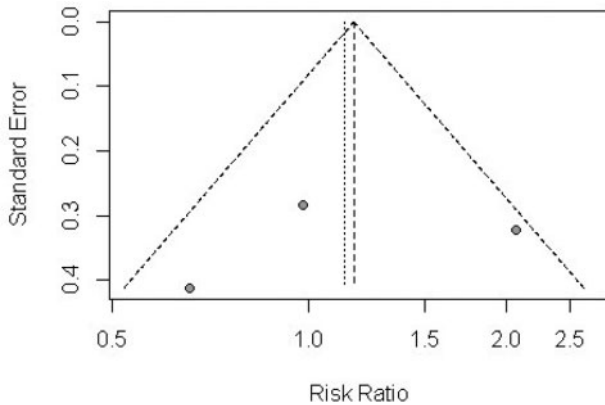
study to another. For example, the rejection rate of objects by yellow warblers in Canada decreased in the order of stars, dumbbells, and eggs, regardless of the timing or size (Guigueno and Sealy 2009). Non-egg-shaped objects such as cylinders, rectangles, and cubes all received higher rejection rates than egg-shaped objects, and the rejection of pointed egg models was more frequent than the rejection for rounded and spherical egg models in both the American robin *T. migratorius* and the grey catbird *Dumetella carolinensis* (Underwood and Sealy 2006). Again, the higher rejection rate of stick-shaped objects compared with egg-shaped objects has been reported in the great reed warbler, red-rumped swallow, and barn swallow (*Hirundo rustica*; Moskát et al. 2003; Yang et al. 2015a). To achieve better comparability, the use of 3D-printed standardized models that can independently quantify and control different dimensions is recommended (Yang et al. 2019c).

In recent years, an increasing number of studies have tried to explore the factors that affect the egg rejection behavior of hosts. Cold weather could cause the egg rejection behavior of parent birds (Shitikov et al. 2019), and the light conditions of the nest sites hinder the efficiency of avian egg recognition to a certain extent (Rutledge et al. 2021). Concurrently, the parasitism rates are also related to the population density of the host and the host spatial distribution (Medina and Langmore 2019). Therefore, taking the hosts' own egg polymorphism into consideration would be more subtle (Yang et al. 2020b). In addition, physiological mechanisms behind these behaviors in ABP are still largely unknown due to the lack of empirical studies. Recently, one study found that mitotane, as a glucocorticoid synthesis inhibitor, could reduce the rejection frequency of foreign eggs in American robins (Abolins-Abols and Hauber 2020). Such new protocols may provide reference suggestions for further studies. Moreover, much of the previous research in this field has largely relied on the use of the receptor noise limited perceptual model, which can reflect avian visual cognition, but detection is restricted when carried out in weak light or other noisy conditions (Aviles 2020). Furthermore, there are similarities between the motor skills and underling behaviors of nest sanitation and egg ejection; however, non-egg-shaped objects were more frequently rejected than egg-shaped objects from nests in most research cases (reviewed in Guigueno and Sealy 2017). Further studies integrating behavior,

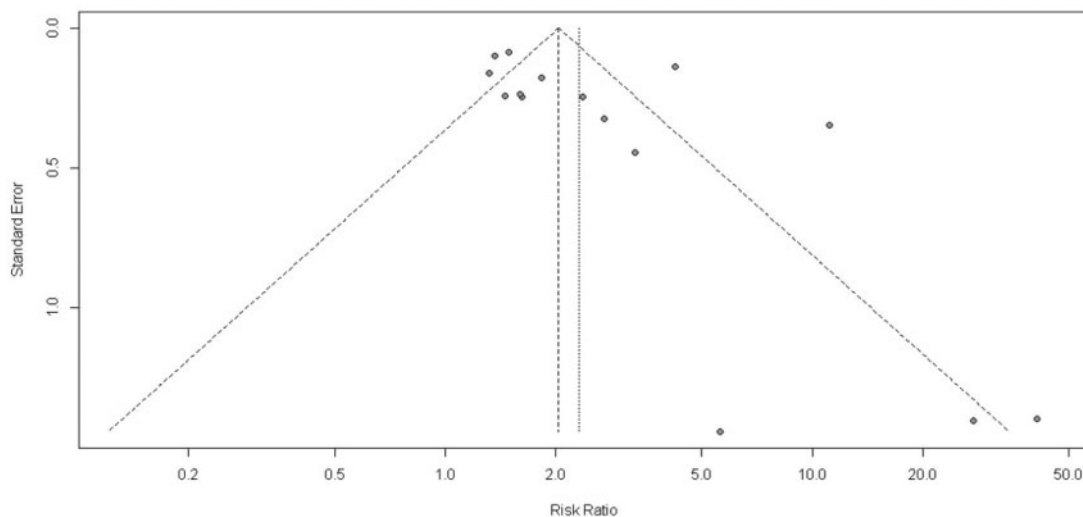**Figure 3.** The funnel plot of three avian species that are studied.

Figure 4. The funnel plot of 15 avian species that are studied (including local species).

Note: (Moskát et al. 2003)[1]; (Luro and Hauber 2017)[2]; (Yang et al. 2015b)[3,5,8,13]; (Su et al. 2018)[4]; This study[6]; (Underwood and Sealy 2006)[7,10]; (Ortega and Cruz 1988)[9,12]; (Guigueno and Sealy 2009)[11]; (Huo et al. 2018)[14]; (Peer 2017)[15].

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physiology, and modeling may be more valuable in exploring the relationship between nest sanitation and egg rejection.

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Author's Contributions

C.Y. conceived and designed the study. Q.L., J.B., and J.W. conducted the investigation in the field. Q.L. and C.Y. analyzed the data and drafted the manuscript. All authors read and approved the final manuscript.

Conflict of Interest Statement

The authors declare that they have no competing interests.

References

Abolins-Abols M, Hauber ME, 2020. Endocrine regulation of egg rejection in an avian brood parasite host. *Biol Lett* 16:20200225.

Antonov A, Stokke BG, Moksnes A, Roskaft E, 2008. Evidence for egg discrimination preceding failed rejection attempts in a small cuckoo host. *Biol Lett* 5:169–171.

Avilés JM, 2020. Avian egg and nestling detection in the wild: should we rely on visual models or behavioural experiments? *Phil Trans R Soc B* 375:20190485.

Bártol I, Moskát C, Karcza Z, Kisbenedek T, 2003. Great reed warblers bury artificial objects, not only cuckoo eggs. *Acta Zool Acad Sci H* 49:111–114.

Bi J, Jiang Y, Yang C, 2020. Breeding ecology of the yellow-bellied warbler *Abroscopus superciliaris*. *Avian Res* 11:41.

Davies NB, 2000 *Cuckoos, Cowbirds and Other Cheats*. London: T & A D Poyser.

Feeney WE, Welbergen JA, Langmore NE, 2014. Advances in the study of coevolution between avian brood parasites and their hosts. *Annu Rev Ecol Evol Syst* 45:227–246.

Feng C, Yang C, Liang W, 2019. Nest sanitation facilitates egg recognition in the common tailorbird, a plaintive cuckoo host. *Zool Res* 40:466–470.

Guigueno MF, Sealy SG, 2009. Nest sanitation plays a role in egg burial by yellow warblers. *Ethology* 115:247–256.

Guigueno MF, Sealy SG, 2012. Nest sanitation in passerine birds: implications for egg rejection in hosts of brood parasites. *J Ornithol* 153:35–52.

Guigueno MF, Sealy SG, 2017. Implications of nest sanitation in the evolution of egg rejection. In: Soler M, editor. *Avian Brood Parasitism: Behaviour, Ecology, Evolution and Coevolution*. Switzerland: Springer. 385–400.

Hoover JP, 2003. Experiments and observations of prothonotary warblers indicate a lack of adaptive responses to brood parasitism. *Anim Behav* 65:935–944.

Huo J, Yang C, Su T, Liang W, Moller AP, 2018. Russet sparrows spot alien chicks from their nests. *Avian Res* 9:12.

Kruger O, 2007. Cuckoos, cowbirds and hosts: adaptations, trade-offs and constraints. *Phil Trans R Soc B* 362:1873–1886.

Lanyon SM, 1992. Interspecific brood parasitism in blackbirds (icterinae): a phylogenetic perspective. *Science* 255:77–79.

Lowther P, 2020. Brood parasitism - host lists. Available from https:// www. fieldmuseum. org/ blog/ brood- parasitism- host- lists.

Luro AB, Hauber ME, 2017. A test of the nest sanitation hypothesis for the evolution of foreign egg rejection in an avian brood parasite rejecter host species. *Sci Nat* 104:14.

Lyon BE, 2003. Egg recognition and counting reduce costs of avian conspecific brood parasitism. *Nature* 422:495–499.

Lyon BE, Eadie JM, 2008. Conspecific brood parasitism in birds: a life-history perspective. *Annu Rev Ecol Evol Syst* 39:343–363.

Lyon BE, Shizuka D, 2017. Context-dependent response to eggs: egg retrieval versus egg rejection in a conspecific brood parasite. *Anim Behav* 132:281–289.

Mann CF, 2017. A taxonomic review of obligate and facultative interspecific avian brood parasitism. In: Soler M, editor. *Avian Brood Parasitism: Behaviour, Ecology, Evolution and Coevolution*. Switzerland: Springer. 61–92.

Medina I, Langmore NE, 2019. Host density predicts the probability of parasitism by avian brood parasites. *Phil Trans R Soc B* 374:20180204.

Moskát C, 2005. Nest defence and egg rejection in great reed warblers over the breeding cycle: are they synchronised with the risk of brood parasitism? *Ann Zool Fenn* 42:579–586.

Moskát C, Hauber ME, 2007. Conflict between egg recognition and egg rejection decisions in common cuckoo *Cuculus canorus* hosts. *Anim Cogn* 10:377–386.

Moskát C, Székely T, Kisbenedek T, Karcza Z, Bártol I, 2003. The importance of nest cleaning in egg rejection behaviour of great reed warblers *Acrocephalus arundinaceus*. *J Avian Biol* 34:16–19.

Ortega CP, Cruz A, 1988. Mechanisms of egg acceptance by marsh-dwelling blackbirds. *Condor* 90:349–358.

Peer BD, 2017. Nest sanitation does not elicit egg ejection in a brown-headed cowbird host. *Anim Cogn* 20:371–374.

Peer BD, Sealy SG, 2004. Correlates of egg rejection in hosts of the brown-headed cowbird. *Condor* 106:580–599.

Poláček M, Griggio M, Bartíková M, Hoi H, 2013. Nest sanitation as the evolutionary background for egg ejection behaviour and the role of motivation for object removal. *PLoS ONE* 8:e78771.

Reel JJ, Underwood TJ, 2019. Egg rejection behavior does not explain the lack of cowbird parasitism on an eastern north American population of red-winged blackbirds. *Avian Res* 10:47.

Roncalli G, Ibáñez-Álamo JD, Soler M, 2016. Size and material of model parasitic eggs affect the rejection response of western bonelli's warbler *Phylloscopus bonelli*. *Ibis* 159:113–123.

Rothstein SI, 1975. An experimental and teleonomic investigation of avian brood parasitism. *Condor* 77:250–271.

Rothstein SI, 1990. A model system for coevolution-avian brood parasitism. *Annu Rev Ecol Syst* 21:481–508.

Ruiz-Raya F, Soler M, 2017. Phenotypic plasticity in egg rejection: evidence and evolutionary consequences. In: Soler M, editor. *Avian Brood Parasitism: Behaviour, Ecology, Evolution and Coevolution*. Switzerland: Springer. 450–471.

Ruiz-Raya F, Soler M, Sánchez-Pérez LL, Ibáñez-Álamo JD, 2015. Could a factor that does not affect egg recognition influence the decision of rejection? *PLoS ONE* 10:e0135624.

Rutledge S, Carr DE, Hauber ME, Hanley D, 2021. Best of a bad job or masters of illusion: do nest light conditions make the eggs of brood parasitic brown-headed cowbirds *Molothrus ater* more similar to the eggs of their hosts? *Ethology* 127:117–124.

Shitikov D, Samsonov S, Makarova T, 2019. Cold weather events provoke egg ejection behaviour in open-nesting passerines. *Ibis* 161:441–446.

Shizuka D, Lyon BE, 2010. Coots use hatch order to learn to recognize and reject conspecific brood parasitic chicks. *Nature* 463:223–226.

Soler M, 2017 *Avian Brood Parasitism, Behaviour, Ecology, Evolution and Coevolution*. Switzerland: Springer.

Soler M, Rodríguez-Navarro AB, Pérez-Contreras T, García-Ruiz JM, Soler JJ, 2019. Great spotted cuckoo eggshell microstructure characteristics can make eggs stronger. *J Avian Biol* 50:e02252.

Stevens M, Hardman CJ, Stubbins CL, 2008. Conspicuousness, not eye mimicry, makes "eyespots" effective antipredator signals. *Behav Ecol* 19:525–531.

Su T, Yang C, Chen S, Liang W, 2018. Does nest sanitation elicit egg rejection in an open-cup nesting cuckoo host rejecter? *Avian Res* 9:27.

Underwood TJ, Sealy SG, 2006. Influence of shape on egg discrimination in American robins and gray catbirds. *Ethology* 112:164–173.

Wang L, Yang C, Møller AP, Liang W, Lu X, 2015. Multiple mechanisms of egg recognition in a cuckoo host. *Behav Ecol Sociobiol* **69**:1761–1767.

Yang C, Chen M, Wang L, Liang W, Moller AP, 2015a. Nest sanitation elicits egg discrimination in cuckoo hosts. *Anim Cogn* **18**:1373–1377.

Yang C, Liang W, Antonov A, Cai Y, Stokke BG et al. 2012. Diversity of parasitic cuckoos and their hosts in China. *Chin Birds* **3**:9–32.

Yang C, Liang W, Møller AP, 2019a. Egg retrieval versus egg rejection in cuckoo hosts. *Phil Trans R Soc B* **374**:20180200.

Yang C, Liang W, Møller AP, 2019c. Using 3D modelling and printing to study avian cognition from different geometric dimensions. *Roy Soc Open Sci* **6**:181938.

Yang C, Wang L, Cheng S, Hsu Y, Moller AP et al., 2020a. Model eggs fail to detect egg recognition in host populations after brood parasitism is relaxed. *Front Zool* **17**:14.

Yang C, Wang L, Liang W, Moller AP, 2015b. Nest sanitation behavior in hirundines as a pre-adaptation to egg rejection to counter brood parasitism. *Anim Cogn* **18**:355–360.

Yang C, Wang L, Liang W, Moller AP, 2019b. High egg rejection rate in a chinese population of grey-backed thrush *Turdus hortulorum. Zool Res* **40**:226–230.

Yang C, Wang L, Moller AP, Liang W, 2020b. Egg polymorphism and highly sensitive egg recognition of cross-phenotypes in rufescent prinias *Prinia rufescens* as effective defenses against brood parasitism. *Integr Zool* **16**:280–285.

Yi T, Sun Y, Liang W, 2020. Egg rejection and egg recognition mechanism of chestnut thrushes *Turdus rubrocanus. Behav Process* **178**:104158.

Zölei A, Hauber ME, Geltsch N, Moskát C, 2012. Asymmetrical signal content of egg shape as predictor of egg rejection by great reed warblers, hosts of the common cuckoo. *Behaviour* **149**:319–406.